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Effect of green and ripe *Carica papaya* epicarp extracts on wound healing and during pregnancy

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ABSTRACT

The traditional use of papaya to treat many diseases, especially skin conditions and its prohibition for consumption during pregnancy has prompted us to determine whether papaya extracts both from green and ripe fruits improve wound healing and also produce foetal toxicity. Aqueous extracts of green papaya epicarp (GPE) and ripe papaya epicarp (RPE) were applied on induced wounds on mice. GPE treatment induced complete healing in shorter periods (13 days) than that required while using RPE (17 days), sterile water (18 days) and Solcoseryl ointment (21 days). Extracts were administered orally (1 mg/g body weight/day) to pregnant mice from day 10 and onwards after conception. 3 ($n = 7$) mice and 1 ($n = 6$) mice given RPE and misoprostol, an abortive drug, respectively experienced embryonic resorption while this effect was observed in none of the mice given GPE ($n = 5$) and water ($n = 5$). The average body weight of live pups delivered by mice given GPE (1.12 ± 0.04 g) was significantly lower than those delivered by mice given water (1.38 ± 0.02 g). In SDS–PAGE, proteins were distributed in three bands (*Mr* range ~8–29 kDa). Band intensity at *Mr* ~28–29 kDa was higher in GPE than in RPE. In contrast, band intensity at low *Mr* (~8 kDa) was found to be higher in RPE than in GPE. Notably, the band corresponding to *Mr* ~23–25 kDa was absent in RPE. These differences in composition may have contributed to the different wound healing and abortive effects of green and ripe papaya.

1. Introduction

Papaya (*Carica papaya* L.) belongs to the family of Caricaceae grown in Australia, Hawaii, Philippines, Sri Lanka, South Africa, India, Bangladesh, Malaysia and a number of other countries in tropical America (OECD, 2005). Other names associated with papaya include tepayas by Kadazan Dusun community in East Malaysia, betik in Peninsular Malaysia, lechosa in Venezuela, pawpaw in Sri Lanka and papali in India (Fasihuddin and Ghazally, 2003). *C. papaya* plant is laticiferous as they contain specialized cells known as laticifers. Lactifiers secrete latex and are dispersed throughout most plant tissues. The papaya-latex is well known for being a rich source of the four cysteine endopeptidases namely papain, chymopapain, glycyl endopeptidase and caricain (Azarkan et al., 2003) and the content may vary in fruit, leaves and roots. Commercially, papaya latex is harvested from fully-grown but unripe fruit. Ripe papaya contains less latex compared to green papaya possibly due to cessation of function or breakdown with age of the latex-producing cells (OECD, 2005).

Papaya fruits, seeds, latex and extracts have been used traditionally to treat various ailments in humans across the world. Papaya seed is found to be a rich source of biologically active isothiocyanate (Nakamura et al., 2007). Unripe pulp of *C. papaya* is rich in carbohydrate and starch (Oloyede, 2005) and also contains cardenolides and saponins that have medicinal value such as cardenolides used in the treatment of congestive heart failure (Schneider and Wolfing, 2004). Papaya latex showed marked *in vivo* efficacy against the rodent gastrointestinal nematode, Heligmosomoides polygyrus (Stepek et al., 2007). The seeds are also used as emmenagogue, thirst quenchers, carminatives or for bites and stings of poisonous insects (Wiart, 2006). In Cambodia, Laos and Vietnam, latex is used to treat eczema and psoriasis (Amenta et al., 2000). Carpaine, an alkaloid with an intensely bitter taste and a strong depressant action on the heart, has been obtained mainly from the leaves, fruit and seeds (Hornick et al., 1978). In Kelantan (a state of Malaysia), the latex of the unripe fruit is used as a poison for criminal purposes (Wiart, 2006).

According to the Indian traditional beliefs, especially among the Karnataka community, banana, papaya, jackfruit, pineapple and all unripe fruits are perceived as 'hot' food and considered to be harmful for pregnant women (Tiwari et al., 1982; Adebiyi et al., 2002). According to the folklore tradition in different parts of Assam and India, *C. papaya* L or *Osbeckia nepalensis* in combination with resin from *Ferula narthex* Boiss are used for abortion (Tiwari et al., 1982). The Kadazan Dusun community in Malaysia use the decoction of papaya root as a means of birth control, preventing

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menstruation and for uterine contractions after birth (Fasihuddin and Ghazally, 2003).

Traditional beliefs and practices regarding papaya have led to several studies. Fruit is the most common part of the papaya plant being analyzed by scientists for its papain activity. Cysteine proteinases are used widely for protein digestion in the food and pharmaceutical industries. The root extract of papaya is effective for the treatment of dysuria as it exerts diuresis in treated rats (Sripanidkulchai et al., 2001). In addition, Oduola et al., 2006 described the antisickling activity of unripe papaya extracts. The antisickling and reversal of sickling activities reside in the ethyl acetate fraction that prevents the sickling of hemoglobin of the sickle cell patients. Oral or intraperitoneal administration of various formulations of *C. papaya* during different phases of pregnancy exerted effects on pregnancy and embryonic development, such as anti-implantation activity, increased post-implantation loss and embryotoxicity (Oderinde et al., 2002; Schmidt, 1995). Most of the studies used different parts e.g., pulp and seeds of unripe (green) papaya. The current study focuses on extracts from epicarp both from green and ripe papaya with the extracts being prepared in PBS at physiologically important temperature of 37 °C. In addition, this study also focuses on the foetal development and survival. Finally, using SDS–PAGE, this paper has shown the difference of protein profiles of different extracts.

2. Materials and methods

2.1. Animal (mice) and samples (papaya)

ICR strain mice aged 7–8 weeks were used as experimental animals, purchased from 'A Sapphire Enterprise', Universiti Putra Malaysia. Animals were housed and handled according to the appropriate guidelines in accordance with the internationally accepted principles for laboratory animal use and care.

Green (unripe) and ripe papaya (*C. papaya* of Hawaian Solo variety) used in this study were collected from The 'Modern Farm' located in Temerloh, Pahang, Malaysia. The fruits (80 green and 80 ripe) were taken before 10.00 am as it is believed that the latex content gradually decreases towards midday.

2.2. Preparation of the extracts

The epicarps from the washed papaya both ripe and green were peeled using fruit peelers to obtain homogenous thickness and dried in a warm room. The dried ground epicarp was mixed with PBS (50 g/L) and kept at 37 °C in an incubator shaker for 6–8 h. The mixture was centrifuged at 200 g at 27 °C for 30 min and the supernatant was collected followed by rotavaporization and finally freeze dried to obtain the final extract in powder form.

2.3. Induced wound and treatment

An area of uniform wound (6 mm) was excised on the back of the body using a biopsy puncher. Prior to incision, the mice ($n = 9$ for each group) were anaesthetized by intraperitonal injection using Nembutal (50 mg/kg body weight). Freeze-dried powder in sterile deionized water (5 mg/ml) was topically applied (10 μL) twice daily on the wound. Parallel groups were treated using sterile deionized water (negative control) and Solcoseryl (a protein-free haemodialysate from calf's blood) ointment (positive control). Diameter of the wound was recorded every 72 h, usually in the morning.

2.4. Oral administration of the extracts

Different groups of pregnant mice were given different extracts orally (gastric intubations) from E10 until delivery where the first day of mating is considered as E0 (embryonic day 0). Mating was confirmed by the presence of vaginal plug. Mice found to be positive for vaginal plug were separated from male counterparts. Afterwards, their body weight was recorded regularly. An increased body weight and distended abdomen by E10 of the mouse were considered as confirmation of pregnancy. Extracts were given orally at 0.5 mg/g body weight/day (twice daily). Parallel groups were given either the commercially available abortive drug, misoprostol (Cytotec®), 0.2 μg/g body weight/day (positive control) or only deionized water (negative control).

2.5. SDS–PAGE and silver staining

Different preparations of extracts were analyzed for their protein content by SDS–PAGE, essentially performed according to Laemmli (1970). Briefly, extracts (10 μg/100 μl) in sample buffer (2-ME and Bromophenol blue) were heated in a boiling water bath for 5 min. 5 μL of the samples thus prepared was loaded into the gel (17.5% polyacrylamide) wells and run at 25 mA/gel using BIO-RAD Mini-PROTEAN ® 3 Cell (Bio-Rad Lab. Inc, Cal, USA). The gel was stained using rapid silver staining. Briefly, the gel was incubated (10 min) in formaldehyde fixing solution with gentle agitation followed by washing with distilled water twice (5 min each) and soaking in sodium thiosulfate for 1 min and another round of washing (20 s each) in distilled water. The gel was then soaked in 0.1% silver nitrate (10 min) followed by washing with distilled water and developed by sodium thiosulfate developer until the bands appeared. Once the bands appeared, the reaction was stopped by the addition of citric acid followed by washing in distilled water.

2.6. Statistical analysis

One-way ANOVA was done by using SPSS software v12.0.1 followed by Tukey's HSD test for post hoc analysis when necessary. A *P*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Green papaya epicarp extract gives faster epidermal wound healing

Epicarp extracts prepared using either green papaya (GPE) or ripe papaya (RPE) were applied twice a day on induced wounds on mice (initial diameter of 6mm) from the first day. The diameter of the wound was recorded every 72 h (Fig. 1A). Mean days required for complete wound healing treated with GPE, RPE, negative control (treated with sterile deionized water) and positive control (treated with Solcoseryl ointment) were 13, 17, 18 and 21 days respectively (Table 1) that confirms significantly faster wound healing by GPE compared to negative control. Treatment with GPE resulted in average wound closure of around 5 mm by day 10, while treatment with RPE and negative control during the same period of time resulted in wound closure of around 4 mm (Fig. 1B).

3.2. Ripe papaya epicarp extracts contribute to embryonic resorption

Embryonic resorption was confirmed by the decreasing body weights and gradually shrinking abdomens of the pregnant mice from E10 onwards after daily administration of the extracts or drugs from E10 as well as the eventual failure to deliver any pups.

3 out of 7 of the mice given RPE experienced embryonic resorption. Mice from the other groups given GPE ($n = 5$) did not experience embryonic resorption as the body weight continued to increase until the day of delivery. None of the mice given water (negative control group) experienced embryonic resorption, while 1 out of 6 mice given abortive drug misoprostol (positive control group) experienced embryonic resorption.

3.3. Green papaya epicarp extracts contribute to premature delivery

Mice given water without any extracts or abortive drugs ($n = 5$) delivered pups at E19. During this experiment, delivery at any earlier day than E19 is considered as premature delivery. Incidence of premature delivery from each group was recorded to identify the influence of papaya epicarp extracts obtained either from green or ripe papaya. Notably, one mouse from the group given GPE ($n = 5$) delivered 5 days earlier than usual i.e. at E14.

3.4. Green papaya epicarp extracts affect survival of the pups

The number of dead pups found on the day of delivery was recorded to identify whether the consumption of the extracts by the mother during pregnancy was embryotoxic. GPE extract reduced the survival of the embryos when compared with negative control group. 77% ($n = 56$) of the total pups delivered by mice given GPE

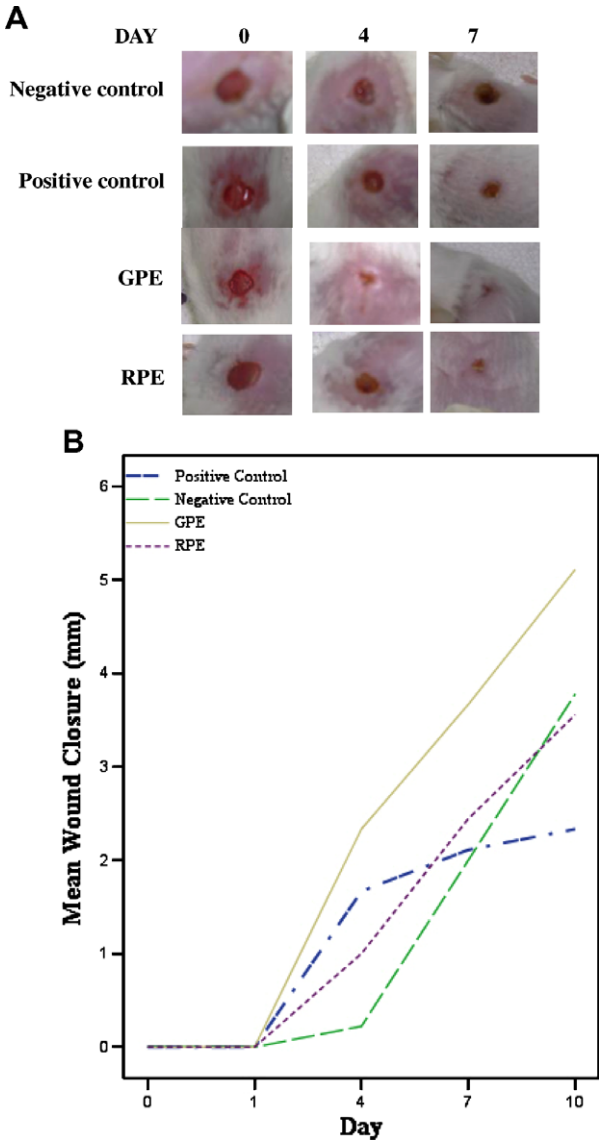

Fig. 1. Rate of wound healing, i.e., reduction of wound diameter per day is faster when treated with green papaya epicarp extract (GPE). (A) Representative photographs of wounds on day 0, 4 and 7 from different groups of mice treated with sterile deionized water (negative control), Solcoseryl (positive control), GPE and RPE. (B) Mean wound closure in mm measured by subtracting the size of wound on each day from the original wound size, i.e., 6 mm.

Table 1
Number (mean) of days required for complete healing of induced wounds

Treatment (concentration)	days required for complete healing (mean ± S.E.M)
Sterile deionized water (negative control)	18 ± 0.94
Solcoseryl Jelly (positive control)	21 ± 0.41
GPE (5 mg/ml)	13 ± 0.15[a]
RPE (5 mg/ml)	17 ± 1.24

[a] Total number of days required for complete wound healing is significantly lower than negative control ($p < 0.05$).

were found dead on the day of delivery, while none of the delivered pups from negative control groups were found dead. However, the number of dead pups delivered by the mice treated with other treatments varied, i.e., 43% ($n = 44$) in RPE and 58% ($n = 52$) in misoprostol-administered mice groups.

Table 2
Consumption of GPE by mother caused significant decrease in body weights of the delivered pups on the day of delivery i.e., at P0

Groups	No. of live pups	Body weight (g) of live pups at P0 mean ± S.E.M
Positive Control	22	1.27 ± 0.06
Negative Control	72	1.38 ± 0.02
GPE	13	1.12 ± 0.04[a]
RPE	25	1.29 ± 0.03

[a] Statistical analysis shows significant decreases in body weight when compared with negative control ($p < 0.05$).

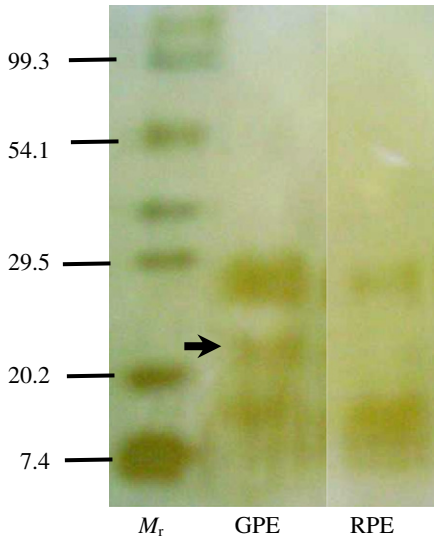

Fig. 2. Silver staining of the SDS–PAGE separated protein profiles obtained from papaya epicarp extract. GPE and RPE represent extracts from epicarp obtained from green and ripe papaya respectively. Proteins are visible only within the range of ~8–29 kDa. A band of protein(s) representing ~23–25 kDa present in GPE, as shown with arrow, is found to be absent in RPE.

3.5. Green papaya extract caused reduction of the body weight of live pups

The body weights of live pups were measured on the day of delivery, usually within 3–4 h after birth. A significant decrease in average body weight was observed in live pups delivered by mice given GPE when compared to live pups from the negative control group (Table 2).

3.6. Protein profiles varies significantly in different extracts

The number of proteins and their respective concentrations differed between extracts obtained either from the green or ripe papaya (Fig. 2), as determined based on the presence or absence and the intensity of bands on gel after silver staining. Three bands were apparent from the GPE and their *Mr* ranged between ~8–29 kDa. The band intensity at *Mr* ~28–29 kDa was more from GPE compared to that from RPE. In contrast, proteins having low *Mr* (in the range of ~8 kDa) were found to be more intense in RPE compared to GPE. Notably, the band corresponding to *Mr* of ~23–25 kDa was absent in RPE while the same band is present in GPE.

4. Discussion

Traditional beliefs and practices have lead to considerable interest in the analyzing of biochemical properties and physiological

effects of bioactive components extracted from various parts of papaya. Traditionally, use of green papaya to treat skin problems and its prohibition for consumption during pregnancy are most commonly referred to and have prompted us to determine whether these responses can be produced by extracts of either green or ripe papaya epicarp. Ripe papaya is most commonly eaten as fresh fruit and green papaya is eaten as a vegetable usually after cooking or boiling.

Researchers have used different parts of the fruit and different extraction solvents (Raji et al., 2006; Schmidt, 1995; Chinoy et al., 1994; Lohiya et al., 1999,2006; Mojica-Henshaw et al., 2003). In the current study, PBS was chosen as the extraction solvent since its isotonic and physiological features are considered favorable for extracting protein-like bioactive components. During extraction, the temperature was never raised above 37 °C to avoid any possible denaturation of the extracted proteins. Epicarp (skin) of the fruit was used in this study as it contains more latex compared to the other parts of the fruit. Furthermore, epicarp of papaya fruit is used as a meal for young pullets (Fouzder et al., 1999). Inclusion of up to 30% papaya peel meal in diet was found to have no effect on the reproductive tract morphometry and some hematological characteristics of female rabbits (Bitto et al., 2006). However, possible effects on other physiological condition were not ruled out.

Wound healing, or wound repair, is the body's natural process of regenerating dermal and epidermal tissue. The sequence of events that repairs the damage is categorized into separate inflammatory, proliferative and maturation phases (Diegelmann and Evans, 2004). Extracts from the epicarp of green papaya used in this experiment have been shown to be beneficial for treatment of wounds. This finding is consistent with the observation that topical application of the unripe fruit papaya promotes desloughing, granulation and healing and reduced odor in chronic skin ulcers (Hewitt et al., 2000). Topical treatment of mush pulp of *C. papaya* containing papain and chymopapain for pediatric infected burns was effective for desloughing necrotic tissue, preventing infection and providing a granulating wound (Starley et al., 1999). In our study, wounds on mice treated with extracts of green papaya (GPE) healed at a faster rate compared to the extracts obtained from ripe papaya epicarp (RPE). Green papaya epicarp contains the proteolytic enzymes, chymopapain and papain, which have antimicrobial (Emeruwa, 1982) and antioxidant properties (Osato et al., 1993). In addition, fermented papaya preparations have antioxidant properties related to both hydroxyl scavenging and iron chelating properties (Imao et al., 1998; Calzuola et al., 2006). Moreover, antioxidant activities of the *C. papaya* decrease the risk of oxidative damage to the tissues. Osato et al. (1993) reported antibacterial and antioxidant activities of green papaya and they correlated the bacteriostatic activity with its scavenging action on superoxide and hydroxyl radicals. These antioxidants are considered to be one of the potential contributors to wound healing (Mahmood et al., 2005).

In SDS–PAGE (17.5% acrylamide), most of the proteins are distributed in three major bands, *M*r of which ranges between ~8 kDa and ~29 kDa (Fig. 2). Band intensity at *Mr* ~28–29 kDa was higher in GPE compared to that in RPE. In contrast, band intensity at low *Mr* (in the range of ~8 kDa) was higher in RPE compared to that in GPE. Notably, band corresponding to *Mr* of ~23–25 kDa was present only in GPE. This band may represent papain, the well-known protein in papaya having *Mr* of ~23.5 kDa that was previously identified to contribute to wound healing (Brenda et al., 1995). However, the current study indicates that besides the papain content of the green papaya, variation in content of other proteins also might be equally important for wound healing.

Extracts were applied by mixing in deionized water to provide moisture in the wound bed; this is essential for the enzymatic activity (Wright and Shi, 2003). Enzymatic debriding agents are typically used in conjunction with moist wound healing and serve as adjuncts to the autolytic debridement process. Exogenous enzymes typically promote a more rapid debridement than allowing the autolytic process to proceed unaided. Papain, a major component of papaya latex is a nonspecific cysteine proteinase that is capable of breaking down a wide variety of necrotic tissue substrates over a wide pH range from 3.0 to 12.0 (Wright and Shi, 2003). This factor may also have contributed to the faster wound healing and was facilitated by the action of the proteinases.

As the papaya fruit ripen, the amount of laticifers cells that produce latex decreases (OECD, 2005). Therefore, ripe papaya contains less latex and other constituents. The amount of wound healing constituents of the latex would be more in GPE than in RPE. Therefore, this may explain why wounds treated with GPE required less time for complete healing compared to the wound treated with RPE.

Inflammation plays a role in fighting infection and inducing the proliferation phase necessary for healing. However, inflammation can lead to tissue damage if it lasts too long. Thus the reduction of inflammation is frequently a goal in therapeutic settings (Diegelmann and Evans, 2004). Wounds treated with solcoseryl ointment showed oedema reflecting persistent inflammation (Fig. 3). In contrast, wounds treated with GPE showed no or reduced oedema. This can be correlated with the faster reduction of the wound size at around 1 mm/day until day 4 (Fig. 1B). Notably, Mojica-Henshaw et al. (2003) reported immunostimulatory and anti-inflammatory actions of *C. papaya* seed extract. Using lymphocyte proliferation assay and complement-mediated hemolytic assay, they found that the crude seed extract and two other bioactive fractions significantly enhanced the phytohemagglutinin responsiveness of lymphocytes. They also concluded that no single compound is likely to be responsible for those activities. In addition, Dawkins et al. (2003) have shown antibacterial effects of *C. papaya* fruit on common wound organisms; however, the effect was independent of stage of fruit maturity.

In the current study, RPE caused a higher percentage of embryonic resorption compared to GPE whereas mice given GPE experienced premature delivery. An increased number and amount of proteins, evaluated from the pattern of bands and its intensities, is observed in GPE compared to that from RPE. Moreover, in silver-stained protein profile, a band representing the protein(s) having *Mr* more than 20 kDa was absent in RPE (arrow in Fig. 1). Notably, *Mr* of the papain, having ability to degrade proteins, is 23 kDa (Dreuth et al., 1968). These differences in protein content and concentrations might have caused the difference in incidence of both the embryonic resorption and premature delivery. However, further studies are required to identify the exact mechanism.

Traditionally, green papaya has been used to achieve abortion (Tiwari et al., 1982). Adaikan and Adebiyi (2004) found that crude papaya latex and its proteinases such as papain and chymopapain are strong uterine contractants, explaining the abortifacient properties of papaya. Consumption of large quantities of unripe papaya

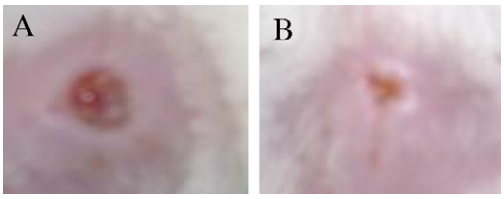

Fig. 3. Oedema formation around the wound after the treatment. (A) Oedema observed in wound treated with Solcoseryl jelly. (B) Oedema has never appeared around the wound treated with GPE. Pictures shown the wound are taken at day 4 during treatment.

fruit and subsequent ingestion of papaya latex can cause uncontrolled uterine contractions leading to abortion depending on the estrogen levels in the tissues that could be due to uterotonic effects of combination of enzymes, alkaloids and other substances instead of the pure papain itself (Cherian, 2000). This conclusion is consistent with the findings of Schmidt (1995) who previously showed that papain alone up to a dose of 800 mg/kg (in rats) neither influences prenatal development nor caused maternal toxicity. In addition, Adebiyi et al. (2002) suggested that unripe papaya fruit may induce miscarriage in susceptible pregnant females as the unripe papaya contains much more latex compared to the ripe papaya.

Phytochemicals in papaya may suppress the effects of progesterone (Gopalakrishnan and Rajasekharasetty, 1978). Adebiyi et al. (2002) found that crude papaya latex induced spasmodic contraction of the uterine muscles similar to oxytocin and prostaglandin-F2α. This is the most probable reason for the abortifacient properties of papaya epicarp extracts since progesterone plays a vital role during pregnancy. During pregnancy, corpus luteum continues to secrete progesterone for the first trimester, after which the placenta becomes the supplier of both progesterone and estrogen. Progesterone prevents further ovulation and relaxes the uterus to prevent the fertilized ovum being dislodged. In the absence of pregnancy, a decline in progesterone level results in shedding off the uterine endometrium and menstruation. The suppression of progesterone will lead to the contraction of uterine smooth muscle and consequently lead to abortion (Paul, 2000).

The number of live pups delivered by the GPE treated groups was significantly lower than the number of pups delivered by other groups (Table 2). Besides, pups delivered by the GPE treated groups had significantly reduced body weights (Table 2). Although, an exact conclusion cannot yet be determined, however, the variation could be due to the difference in protein content and concentration of the extracts. Contrary to our observation, chloroform extract of papaya seed caused significant decrease in litter size but not the foetal weight (Raji et al., 2006). The difference between this and the current study may be due to the difference in bioactive components extracted using different solvents. However, the exact cause of abortive effects remains inconclusive. Nevertheless, it can be hypothesized that the mechanism of abortion by GPE and RPE may be different due to differences in composition.

5. Conclusion

Not contrary to traditional beliefs we have observed that papaya, in particular green papaya, has potential wound healing effects. Again, both the green and ripe papaya epicarp extract influence different aspects of foetal growth and pregnancy.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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